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Demonstration of the Stereospecific Action of Microorganisms in Soil by Gas Liquid Chromatography

Many microorganisms and enzymes are endowed with the ability to preferentially metabolize, incorporate, or chemically alter one antipode of a racemic substrate while leaving the other antipode intact. This intrinsic property can form the basis of experiments aimed at the detection of biological agents in planetary soils. A high-sensitivity gas-liquid chromatographic technique (1) has been employed to monitor the stereospecific consumption of several racemic amino acid substrates in a prototype terrestrial experiment.

In a typical assay, soil (10 gm), the racemic amino acid substrate (10 mg), and distilled water (10 ml) were shaken at room temperature. From time to time aliquots (approx. 1 ml) were removed and diluted with water (10 ml). The soil was centrifuged down and the supernatant solution lyophilized. After esterification with thionylchloride-methanol (0.4 ml in 5 ml) (2) and evaporating, the residue was coupled with *N*-trifluoroacetyl-L-prolyl chloride (0.2 m*M*) in methylene chloride (2 ml) (1) in the presence of triethylamine (0.06 ml). After washing (H_2O) and drying (Na_2SO_4), a part of the solution ($\sim$2 μl) was injected into the gas chromatograph. By computing the peak areas of the two diastereoisomers, a fast sensitive recording of unused D/L amino acid concentration could be obtained (Table 1, Fig. 1).

Our results show that the L-antipodes of the substrates are preferentially attacked, but that different amino acids are used at different rates. The observation that the stereospecific action is lost after heat sterilization of the soils confirms that a biological process is involved.

TABLE 1

SUSCEPTIBILITY OF DL-AMINO ACIDS TO MICROORGANISMS IN SOILS

Soil sample	DL-Amino acid	Unused D/L amino acid concentration after period of incubation (hr)[a]													
		0	2	4	6	8	10	12	14	16	18	20	22	24	48
Bowers clay[b]	Proline	1.0		1.0		1.1				1.2	1.9	3.0	6.8	9.2	
Bowers clay[c] sterilized	Proline	1.0								1.0				1.0	
Bowers clay	Glutamic acid	1.0		1.0		1.1		1.3		1.3	1.5	2.5	2.7	5.0	
Bowers clay[c] sterilized	Glutamic acid	1.0						1.0						1.0	
Stanford soil[d]	Glutamic acid	1.0						1.8			6.7			16.0	
Stanford soil[c] sterilized	Glutamic acid	1.0						1.0						1.0	
Stanford soil	Aminobutyric acid	1.0				1.1								2.9	4.7

[a] GLC analyses were carried out on a Wilkens 600C Aerograph using a 5′ × 1/8″ column (0.5% EGA on Chromosorb W). During the analyses the nitrogen flow was 46 ml/min and the oven temperature was programed from 140° to 200°C at a rate of 4°/min. Under these conditions the retention times (min) for the *N*-TFA-L-prolyl derivatives were D-valine (7.6), L-valine (8.9), D-aminobutyric acid (9.0), L-aminobutyric acid (10.5), D-proline (13.0), L-proline (14.2), D-glutamic acid (20.2), and L-glutamic acid (21.4).

[b] Soil collected at Moffett Field, California, and characterized by NASA Ames. The organic nitrogen analysis was 1,435 ppm and the organic carbon was 6,380 ppm. The soil had a pH of 6.08.

[c] Soil samples were sterilized by heating at 135°C for 24 hr.

[d] Garden soil collected at Stanford in December, 1965.

While the exact nature of the biological system responsible for the stereospecific attack is as yet not known, the kinetics of the experiment (Fig. 1) suggest an exponential increase of activity, which would be consistent with growth of microorganisms.

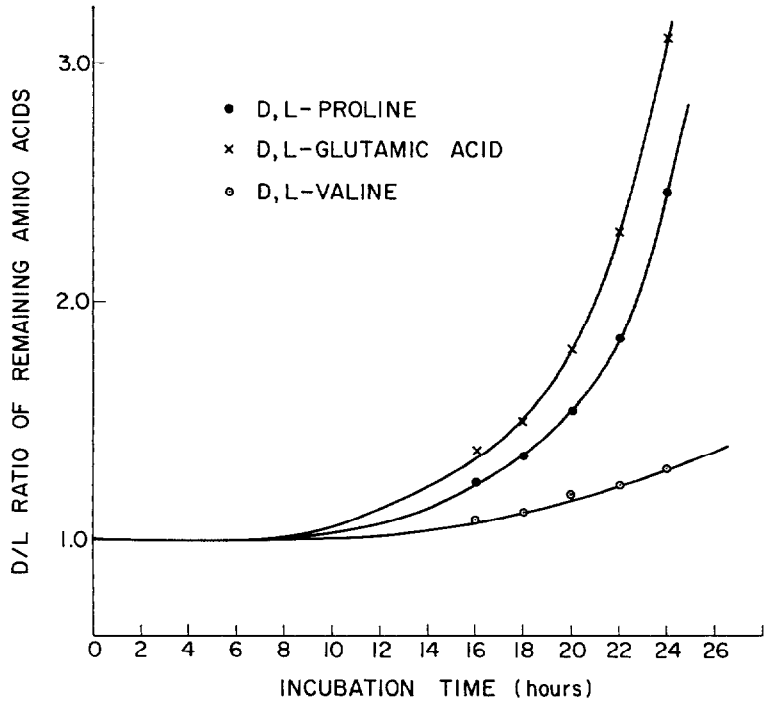

FIG. 1. Susceptibility of a mixed DL-amino acid substrate to microorganisms in Bowers clay.

The experiment has also been implemented with similar results by inoculating soil *in situ* with DL-amino acids, and removing samples from the vicinity of inoculation at various times for differential assay of the enantiomers. This design would be especially attractive for a planetary landing mission.

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Erratum

Analytical Biochemistry **14**, 28 (1966), "Lipolysis of Fluorescein and Eosin Esters. Kinetics in Hydrolysis." The authors of this paper were George G. Guilbault and E. V. Crabtree. Mrs. Crabtree's name was inadvertently omitted.

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